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The effect of temperature on the GABA-induced chloride current in isolated sensory neurones of the frog

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1 The effect of temperature on the kinetics of the activation and inactivation phases of γ-aminobutyric acid (GABA)-induced Cl^- current (I_{Cl}) was examined in frog isolated sensory neurones.

2 The peak I_{Cl} was reversibly reduced on changing the temperature and temperature-dependent coefficients were shown to exist, with the highest Q_{10} (1.58) occurring between 5–15°C.

3 At both room temperature (20°C) and 10°C, the GABA dose-response curve was sigmoidal with a Hill coefficient of 2 and half-maximal responses to GABA, K_d, of 1.3×10^{-5} M and 1.1×10^{-5} M, respectively. Thus, indicating no change in the binding affinity of GABA when the temperature was decreased.

4 At GABA concentrations greater than 10^{-5} M, both the activation and inactivation phases of the GABA-induced I_{Cl} consisted of double exponentials, fast and slow components respectively, in the temperature range of 10 to 30°C.

5 The fast (τ_{af}) and slow (τ_{as}) activation time constants decreased with an increase in temperature and increased with a reduction in temperature. With an increased temperature, the reduction in peak I_{Cl} was due to a reduction in the slow time constant with no significant change in the fast time constant.

6 Both the fast (τ_{if}) and slow (τ_{is}) inactivation time constants were also increased by cooling to 10°C; heating to 30°C had little effect.

7 The concentration-dependence (10^{-5} to 10^{-3} M) of the slow activation (τ_{as}) and inactivation (τ_{is}) time constants was unaltered by the change in temperature. Similarly, the lack of concentration-dependence shown by the fast activation (τ_{af}) and inactivation (τ_{if}) time constants was unaltered by the temperature change.

8 From recordings made with 'inside-out' patches, the probability of opening of the GABA-induced Cl^- channels showed a marked increase with cooling to 10°C compared to room temperature (20°C), with no change in channel conductance.

9 The change in the GABA-induced I_{Cl} at different temperatures is, therefore, not due to changes in binding but to subsequent channel activation. Possible mechanisms whereby this occurs are discussed.

Introduction

γ-Aminobutyric acid (GABA) is an important inhibitory neurotransmitter in the vertebrate nervous system whose response is mediated primarily, if not exclusively by Cl^- current (I_{Cl}) (Krnjevic, 1974). However, little is known of the effect of temperature on the kinetic properties of the GABA-induced I_{Cl}.

At the crayfish neuromuscular junction, GABA mediates an inhibitory postsynaptic current (i.p.s.c.) and there is a prolongation of the rise time and the decay phase of the i.p.s.c. with a reduction in temperature (Dudel, 1977; Onodera & Takeuchi, 1979). A similar observation was made on cat dorsal root ganglion cells to ionophoretically applied GABA, including a depression of the peak amplitude of the response (Gallagher *et al.*, 1983). In contrast, GABA-mediated dorsal root responses in frog spinal cord were increased on cooling to 5°C (Nicoll, 1978).

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Using pressure application of GABA and fluctuation analysis on cultured spinal neurones of the mouse, Mathers & Barker (1981) demonstrated that the GABA-activated Cl channel conductance increased and the mean open time of these channels decreased at higher temperatures.

There are, however, problems in examining temperature effects *in vivo* on the kinetics of a GABA-mediated synaptic response, such as release and subsequent receptor activation. There are also limitations in the use of ionophoretic and pressure application techniques in analysing the channel kinetics of a GABA-receptor interaction, such as application time and actual concentration of the ejected dose at the receptor site. Moreover, it is desirable to examine the temperature-dependence of a GABA-induced I_{Cl} in the absence of contamination by other ionic currents. Also the rapid application of a known concentration is needed to examine temperature effects on the kinetics of this reaponse quantitatively.

In frog isolated sensory neurones, by use of a combination of voltage clamp, internal perfusion and a rapid drug application technique, the 'concentration-clamp', the GABA-induced I_{Cl} was demonstrated to have both activation and inactivation phases consisting of double exponentials (Akaike *et al.*, 1986). Therefore, using these techniques, we examined the effects of temperature on the kinetics of the GABA-induced I_{Cl} in these cells.

Methods

Preparation

Experiments were performed on dorsal root ganglia dissected from the American bull-frog (*Rana catesbiana*). Following removal of calcified tissue surrounding the ganglion, the capsules enveloping the ganglion masses were digested in Ringer solution containing 0.3% collagenase and 0.05% trypsin for approximately 18 min at 37°C. A continuous gentle shaking of the ganglia was achieved by bubbling the bathing medium with 95% O_2 and 5% CO_2 to facilitate enzymatic digestion. Thereafter, single cells were mechanically isolated from the ganglion with finely polished pins under a microscope and left in a medium consisting of equal parts of Ringer solution and Eagle's minimum essential medium (Nissui, Japan) for 3 to 12 h at room temperature (approx. 20°C).

Solutions

To isolate the I_{Cl} from all other ionic currents such as Na^+, K^+ and Ca^{2+} currents, Na^+, K^+ and Ca^{2+} in both internal and external test solutions were replaced with tris(hydroxymethyl)aminomethane (Tris), Cs^+ and Mg^{2+}, respectively. The isolated nerve cell body was perfused with Na^+-, K^+- and Ca^{2+}-free external and internal solutions containing 120 mM Cl^-. The ionic compositions of solutions (in mM) were: internal solution, CsOH 10, aspartic acid 10, TEA-Cl 25, CsCl 95, EGTA 2.5, pH 7.2; external solution, Tris-Cl 90, CsCl 2, $MgCl_2$ 5, TEA-Cl 18, glucose 5, pH 7.4. The pH of all solutions was adjusted with Tris-base and/or N-2-hydroxyethylpiperazine-N'-2-ethane sulphonic acid (HEPES).

Suction pipette

Several single cells in a culture dish were suctioned into a tapered capillary with a fire polished tip (diameter of approximately 500 μm) and transferred into another culture dish, 3 cm in diameter. Single cells were clearly visible under binocular magnification of 80 ×. A suction pipette technique was used for voltage-clamp and internal perfusion (Ishizuka *et al.*, 1984; Akaike *et al.*, 1986). A Pyrex-glass tube with a 3 mm outer diameter was pulled to a shank length of 2.5 to 3.0 mm. The pipette tip was cut to an outer diameter of about 40 μm and fire-polished to give an outer diameter of about 7 μm. Part of the individual neurone (30 to 40 μm diameter) was aspirated by the suction pipette with a negative pressure of about 3 cmHg. The aspirated membrane either ruptured spontaneously or was ruptured by applying 5 to 20 nA square wave pulses of depolarizing current (10 to 50 ms). Thereafter the neurone was internally perfused at a constant flow rate of 1 ml min^{-1}. Adequacy of internal perfusion with the present suction pipette technique was evaluated by determining how close the reversal potential for the GABA-induced Cl^- response (E_{GABA}) was to the Cl^- equilibrium potential (E_{Cl}) of about +4 mV, calculated from the Nernst equation based on the Cl^- activities in the internal and external solutions. Here, the Cl^- activities were estimated by using a F1012Cl Cl^- electrode connected to an ION85 Ion Analyzer (Radiometer A/S, Copenhagen).

Electrical measurements

The membrane potential was measured through an Ag-AgCl wire in a Ringer-agar plug mounted on the suction pipette holder. The reference electrode was also an Ag-AgCl wire in a Ringer-agar plug. The resistance between the suction pipette filled with standard internal solution and the reference electrode was 200 to 300 kΩ. Both electrodes were led to a voltage-clamp circuit and the membrane potential was controlled by a single-electrode voltage-clamp system switching at a frequency of 10 kHz and passing current for 36% of the cycle (Ishizuka *et al.*, 1984). Clamp currents were measured as the voltage

drop across a 10 MΩ resistor in the feedback path of a headstage amplifier. In this system, the suction electrode could carry time-averaged currents exceeding 100 nA at a switching frequency of 10 kHz without showing signs of polarization or other artifacts. Both current and voltage were monitored on a digital storage oscilloscope (National, VP-5730A) and simultaneously on an ink recorder (Rikadenki, R-22), and stored on an FM data recorder (TEAC, MR-30). Fast and slow time constants of activation and inactivation phases were determined by curve peeling from tape recorded data through the use of a program run on a computer (NEC, PC-9801E).

Single channel recordings

All recordings were obtained from 'inside-out' preparations ($n = 4$) using a conventional patch clamp technique as described previously (Yasui *et al.*, 1985). The external (patch pipette) and internal solutions, each containing 120 mM Cl^-, were the same as those used in the whole cell preparation. Recording bandwidth was d.c. to 10 kHz and holding potential (V_H) was −50 mV.

Application method

The 'concentration-clamp' method was developed to enable an extremely rapid application or 'switching' of the external solution containing agonists such as GABA (Akaike *et al.*, 1986). The cell-attached tip of the suction pipette was inserted into a plastic tube through a circular hole 500 μm in diameter. The lower end of this tube could be directly exposed to the external test solution by vertically moving a stage holding solution containing dishes. A negative pressure of −3 cmHg applied to the upper end of the plastic tube was controlled by an electromagnetic valve, driven by 24 V d.c. supplied by a stimulator (Nihon Kohden, SEN-7103). The speed of the solution exchange could be estimated from the time lag of the current shift under voltage-clamp conditions. Solution temperatures were constantly monitored using a thermistor probe (Nihon Kohden, MGA-219) at the experimental set-up; solutions were maintained at the desired temperatures in a cooling/heating chamber (Rikadenki, UA-100). Cold or warm solutions were rapidly exchanged to the recording stage with constant temperature monitoring. From room temperature, the cell was exposed to agonist-free solution at the temperature under investigation (e.g., 10°C) for 15–30 s before applying the cold GABA solution. For washout, the cell was re-exposed to agonist-free solution at the temperature under investigation (e.g., 10°C) followed by application of solution at room temperature (20°C). A slight current shift was produced at 10°C with a time constant of 162 ms as compared with the response to

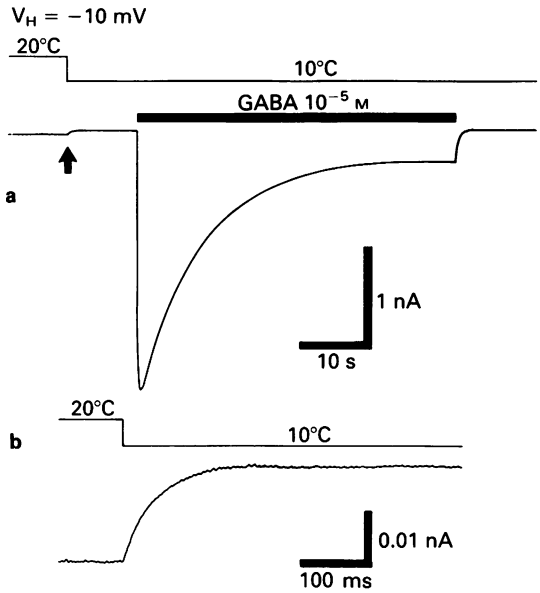

Figure 1 (a) Current shift (arrow), of frog isolated sensory neurones, in response to the switching of the external solution from room temperature (20°C) to 10°C followed by a 10^{-5} M GABA-induced I_{Cl} under voltage-clamp. (b) Expansion of the current shift in (a) (arrow) illustrating the rapid time course of the effect.

10^{-5} M GABA (Figure 1a (arrow) and b). This current shift recovered completely to the control level recorded before cooling when the temperature was raised from 10 to 20°C. There was no difference in the time constants of the current shifts with onset and offset of cooling. No appreciable current shift was observed at 30°C. Cell viability was continuously monitored by recording the peak I_{Cl} to 10^{-5} M GABA; all applications of GABA were at 10 min intervals. The time constant of the effect of GABA on these cells was a few ms (Akaike *et al.*, 1986). Data were normalized to the peak current induced by 10^{-5} M GABA except where noted.

Current recording without 'Cl⁻ redistribution'

A continuous application of GABA elicits an 'apparent' desensitization consisting of both 'real' GABA receptor-inactivation and Cl^- redistribution across the soma membrane which results in a decreased I_{Cl} due to a decrease in the driving force (ΔV_H). The ΔV_H is the potential difference between the holding membrane potential (V_H) and Cl^- equilibrium potential (E_{Cl}). As a consequence of this redistribution, E_{Cl} gradually shifts the GABA equilibrium potential (E_{GABA}) (Adams & Brown, 1975; Mayer *et al.*, 1983; Akaike *et al.*, 1987). The shift of E_{GABA}

depends upon the 'Cl^- redistribution', which in turn is voltage-, time- and concentration-dependent, and could be completely prevented by the following treatments: (a) continuous perfusion of the isolated sensory neurone with external and internal solutions containing high intracellular Cl^- such as 120 mM, and (b) maintenance of ΔV_H within 15 mV. All recordings of GABA-induced inward I_{Cl} in the present experiments were made in Na^+-, K^+- and Ca^{2+}-free external and internal solutions containing 120 mM Cl^-, respectively. In addition, V_H was kept at -10 mV which gives a ΔV_H of about 15 mV since E_{Cl} is $+3.4$ mV.

The temperature coefficient, Q_{10}, was used to characterize I_{Cl} as described by Kimura & Reeves (1979):

$$Q_{10} = \frac{X_2(10/T_2 - T_1)}{X_1} \quad (1)$$

where X_1 is the value of the experimental parameter at the lower absolute temperature T_1 and X_2 is that at the higher absolute temperature T_2.

All values are expressed as the mean ± standard error of the mean (s.e.mean).

Results

GABA application to internally perfused sensory neurones of the frog induced a peak inward I_{Cl} which inactivated to a steady-state plateau current during the continuous application of GABA (Figure 1a). At concentrations greater than 10^{-5} M, both activation and inactivation phases show a double exponential at room temperature (Akaike *et al.*, 1986).

Preliminary experiments on the GABA-gated I_{Cl} indicated a working temperature range of between 10 to 30°C where the cells showed good recovery at room temperature, 20°C (RT). Although responses could be obtained at temperatures as low as 5°C and as high as 40°C, the cells deteriorated and did not recover well. Thus, the majority of these experiments were conducted over the temperature range of 10 to 30°C.

A typical 2×10^{-5} M GABA-gated inward current at RT is shown in the centre panel of Figure 2a. Activation of the inward current led to a peak inward I_{Cl} which inactivated to a steady-state plateau current with continuous GABA application. Termination of the GABA application led to a rapid return to the base line level.

In all experiments, GABA was applied continuously until a full steady-state plateau current was obtained. From the same cell, the inward I_{Cl} at 10 and 30°C is also shown (Figure 2a). At both 10 and 30°C, peak I_{Cl} was decreased compared to control at 20°C. At 10°C, the inactivation and the onset of steady-state current appeared to be slower compared to those at RT, as observed by the increased GABA application time. In contrast, at 30°C, although peak I_{Cl} was slightly reduced, the inactivation and the onset of the steady-state current were accelerated as compared with the control at RT. In addition, the amplitude of the steady-state current remaining after inactivation was somewhat greater at low temperatures than at high temperatures. As shown in Figure 2b, a bell-shaped curve was obtained when peak I_{Cl} was plotted against temperature, thus illustrating that with both low and high temperatures, there is an overall decrease in the amplitude of the I_{Cl}. The I_{Cl} showed the highest sensitivity to tem-

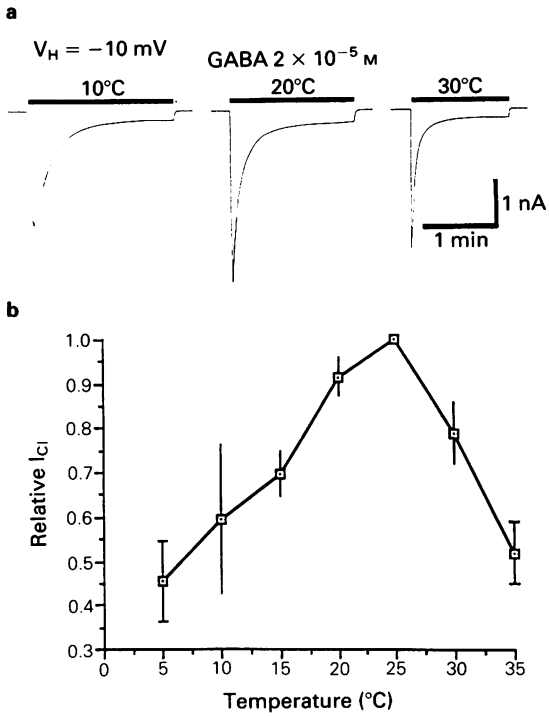

Figure 2 (a) A 2×10^{-5} M GABA-induced I_{Cl} in frog isolated sensory neurones, at the three principal working temperatures used in the study; room temperature (RT) = 20°C and holding potential (V_H) = -10 mV. All recordings were obtained from the same cell. Note the differences in the application times of GABA needed to achieve a steady-state and the decrease in peak I_{Cl} at 10 and 30°C compared to RT. Inactivation of I_{Cl} was more rapid and consequently the onset of steady-state was faster at 30°C than at 10°C. (b) Peak I_{Cl} plotted between 5 and 35°C gives a bell-shaped curve with a decreased I_{Cl} occurring on changing the temperature from RT (20°C). The exception was a small increase at 25°C. Each point is the mean of 6–8 cells and the vertical lines indicate s.e.mean.

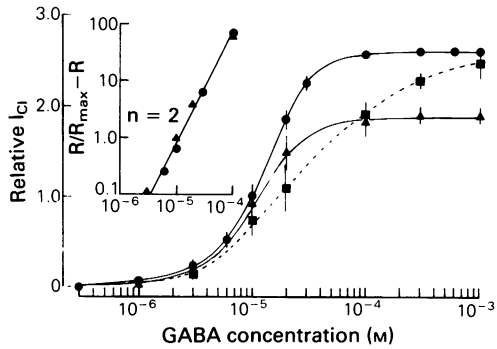

Figure 3 GABA dose-response curves at 10 (▲), 20 (●) and 30 (■) °C. Each point is the mean of 4–11 cells. The vertical lines show s.e.mean. Peak currents induced by GABA at different temperatures were normalized to the peak current induced by 10^{-5} M GABA. Theoretical curves for the GABA responses at 10 and 20°C (RT) were drawn according to equation (2) (see Results) using n = 2 shown in inset and $K_d = 1.1 \times 10^{-5}$ M and 1.2×10^{-5} M for 10 and 20°C, respectively. Note the reduction in the efficacy but not affinity of GABA at 10°C compared with 20°C. At 30°C, experimental data points could not be well fitted according to equation (2) and were fitted by eye.

perature, with a Q_{10} of 1.58, between 5–15°C (Q_{10} = 1.26 between 15–25°C) and the lowest sensitivity between 25–35°C, Q_{10} = 0.52.

Dose-response relationship for GABA-induced I_{Cl}

The GABA-induced inward I_{Cl} increased in a sigmoidal fashion over the dose range 3×10^{-7} to 10^{-3} M at RT (20°C). At concentrations greater than 6×10^{-6} M, I_{Cl} initially increased and subsequently decreased as a result of inactivation which was most conspicuous at concentrations greater than 10^{-5} M (Akaike *et al.*, 1986). Figure 3 shows the peak I_{Cl} values as a function of GABA concentration at 10, 20 and 30°C. The GABA dose-response curves were normalized to the peak I_{Cl} induced by 10^{-5} M GABA. The two continuous theoretical lines at 10 and 20°C were drawn according to the following equation:

$$I = I_{max} \frac{C^n}{C^n + K_d^n} \qquad (2)$$

where I is the observed GABA-induced I_{Cl}, I_{max} the maximum value of I_{Cl}, C the GABA concentration, K_d, a constant corresponding to the concentration of drug producing a half-maximum response and n the Hill coefficient. The latter was derived by linear regression analysis of all points from 6×10^{-6} to 10^{-4} M GABA; at both 10 and 20°C (RT) the Hill coefficient (n) was 2. The K_ds for GABA were

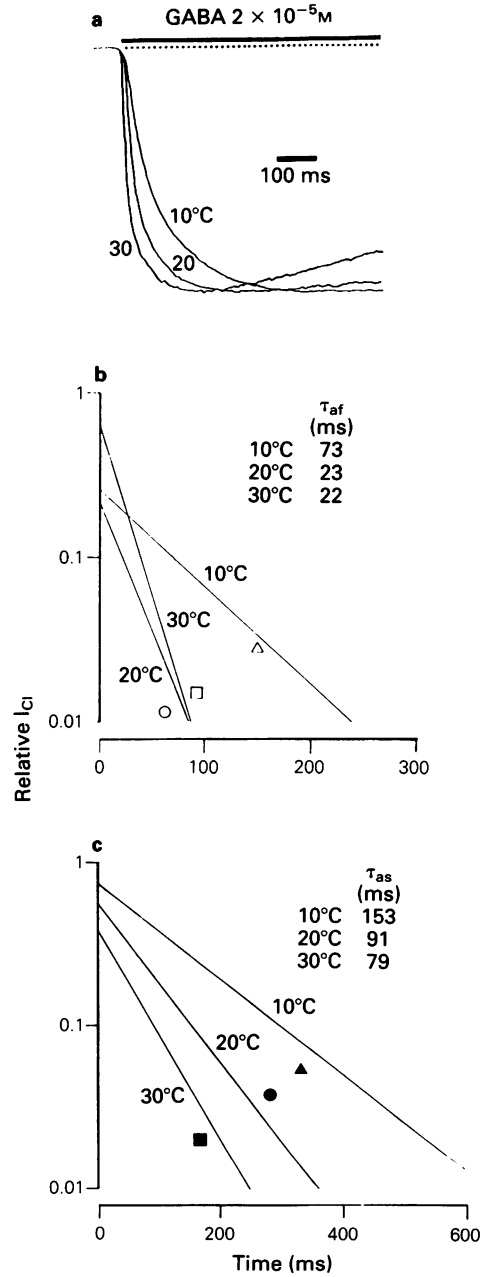

Figure 4 Activation time constants of 2×10^{-5} M GABA-induced I_{Cl} on the same cell at 10, 20 and 30°C. (a) Superimposition of the peak I_{Cl} at 10, 20 and 30°C. The peak currents induced by GABA at 10 and 30°C were normalized to that at 20°C (RT). (b and c) Semi-logarithmic plots of the activation phase of peak I_{Cl}. In this cell, both the fast (τ_{af}) and slow (τ_{as}) time constants were decreased by heating to 30°C and increased by cooling to 10°C.

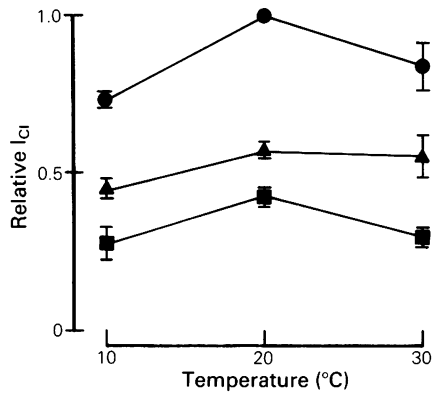

Figure 5 Temperature effect on the contribution of the fast (▲, $Peak_f$) and slow (■, $Peak_s$) activation time constants to the total peak I_{Cl} (●, $Peak_t$) induced by GABA 2×10^{-5} M. Each point is the mean of 4–8 cells. Vertical lines show s.e.mean. The reduction of $Peak_t$ at 10°C is due to a reduction of both $Peak_f$ and $Peak_s$. The reduction of $Peak_t$ at 30°C is due primarily to a reduction of $Peak_s$.

1.1×10^{-5} M and 1.3×10^{-5} M at 10 and 20°C, respectively. These theoretical curves agreed well with the experimental points at 10°C and 20°C.

Although the efficacy of GABA in inducing a peak inward I_{Cl} is reduced at the lower temperature (10°C), identical Hill coefficients (n = 2), similar K_ds and maximum saturating concentrations were obtained. Thus, the affinity of GABA for its receptor is unchanged at lower temperatures in these cells. However, at 30°C, the experimental data points could not be fitted well according to equation (2) and were fitted by eye. As shown in Figure 3, the curve obtained at 30°C deviates from the theoretical curve at concentrations greater than 2×10^{-5} M. Normally at room temperature, with increasing GABA concentrations, the activation phase of the peak inward I_{Cl} becomes more rapid (Akaike *et al.*, 1986). At 30°C the activation phase of the peak inward I_{Cl} became very rapid making extrapolation of the peak inward I_{Cl} extremely difficult and somewhat inaccurate.

Kinetic properties of activation and inactivation of GABA-induced I_{Cl}

At concentrations greater than 10^{-5} M GABA, the time course of the activation of the GABA-induced I_{Cl} consisted of double exponentials, a fast (τ_{af}) and slow (τ_{as}) component. Figure 4a illustrates the activation phase of a cell at the three temperatures, at a fast sweep speed, for 2×10^{-5} M GABA in which the peak I_{Cl} at 10 and 30°C was normalized to that at RT. At 10°C, there was a slowing or decrease in the

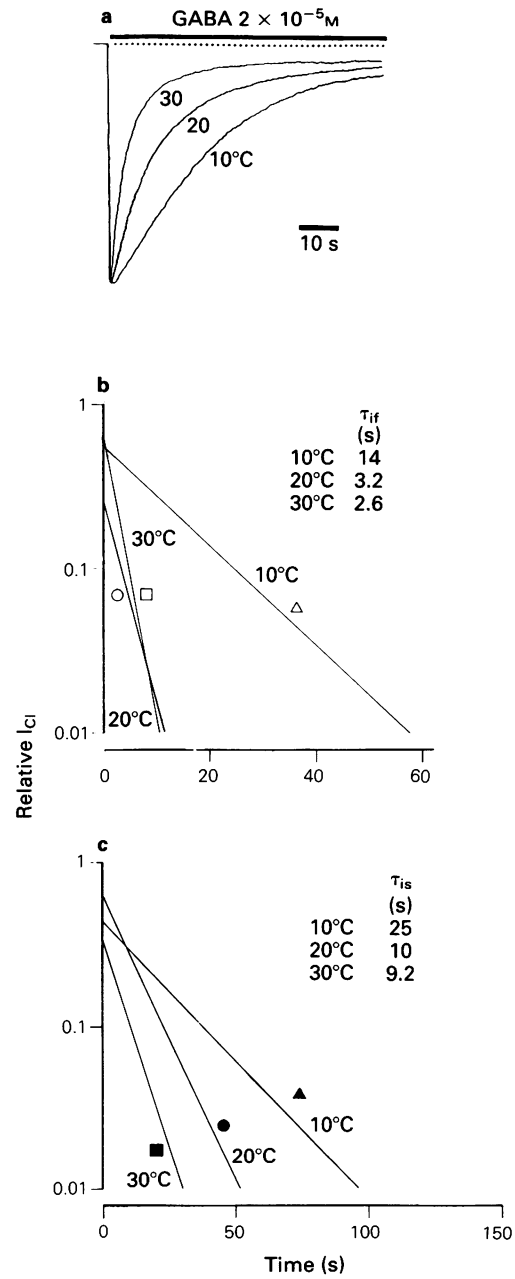

Figure 6 Inactivation time constants on a cell, induced by GABA 2×10^{-5} M, at 10, 20 and 30°C. (a) Superimposition of the traces at the three different tempertures. The figure illustrates a marked reduction in inactivation at 30°C. The peak currents at 10 and 30°C were normalized to that at 20°C. (b and c) Semilogarithmic plots of the fast (τ_{if}) and slow (τ_{is}) inactivation time constants. Both τ_{if} and τ_{is} were decreased with heating to 30°C and markedly increased with cooling to 10°C.

activation phase and a decrease in peak I_{Cl} amplitude. Conversely, at 30°C, there was an increase in activation speed compared to that at the control temperature of 20°C. Figure 4, b and c, also shows the time courses for τ_{af} and τ_{as} at 10, 20 and 30°C. The extrapolation toward zero time of the slow exponential in a semilogarithmic plot gave the time constant of the slow component while the time constant of the fast component was obtained by subtracting the slow component from the total. τ_{af} was markedly retarded at 10°C with a 300% reduction in activation speed (23 versus 73 ms) but there was no effect on τ_{af} at 30°C (Figure 4b). Similarly, τ_{as} was also reduced by 168% at 10°C (Figure 4c), whereas at 30°C, τ_{as} showed an increase in activation speed.

Figure 5 illustrates the effect of changing the temperature on the total peak I_{Cl} ($Peak_t$) and the respective contribution to this current of the fast activation ($Peak_f$) and slow activation ($Peak_s$) time constants of the cells. At all three temperatures, $Peak_f$ accounted for a greater contribution than $Peak_s$ to $Peak_t$. Overall, there was a reduction in $Peak_t$ at both 10 and 30°C, this reduction being somewhat greater at 10°C. $Peak_f$ was virtually unchanged at 30°C compared to a reduction at 10°C. In contrast, $Peak_s$ was reduced almost equally at both 10 and 30°C. At room temperature (20°C), the fast activation component contributed more to the total I_{Cl} than the slow component. This ratio remained the same at 10°C but was altered at 30°C. At 30°C, the relative contribution of the fast component ($Peak_f$) to total peak I_{Cl} ($Peak_t$) increased due to a reduction of the current in the slow activation phase ($Peak_s$).

At concentrations greater than 10^{-5} M GABA, the time course of inactivation of the GABA-induced I_{Cl} is described by double exponentials, a fast (τ_{if}) and a slow (τ_{is}) component (Akaike *et al.*, 1986). The effect of temperature on τ_{if} and τ_{is} induced by 2×10^{-5} M GABA is illustrated in Figure 6, in which the peak currents at 10 and 30°C are normalized to that at 20°C (RT). The increase in the time course of inactivation with cooling is shown in Figure 6b and c. Both τ_{if} and τ_{is} were markedly increased by cooling to 10°C; τ_{if} was increased by 437% (14 s) compared with RT (3.2 s) and τ_{is} by 250% (25 s) compared to RT (10 s). In contrast, heating to 30°C had a small effect on the rate of inactivation, an 18% and 8% decrease in τ_{if} and τ_{is} were observed, respectively.

The activation and inactivation time constants plotted as a function of GABA concentration, for the three temperatures studied in all the cells, are shown in Figure 7. As previously demonstrated by Akaike *et al.* (1986), both τ_{as} and τ_{is} showed a concentration-dependence whereas τ_{af} and τ_{if} did not. This concentration-dependence was unaffected by either temperature change. The fast activation time constant, τ_{af}, was little affected by cooling to 10°C or

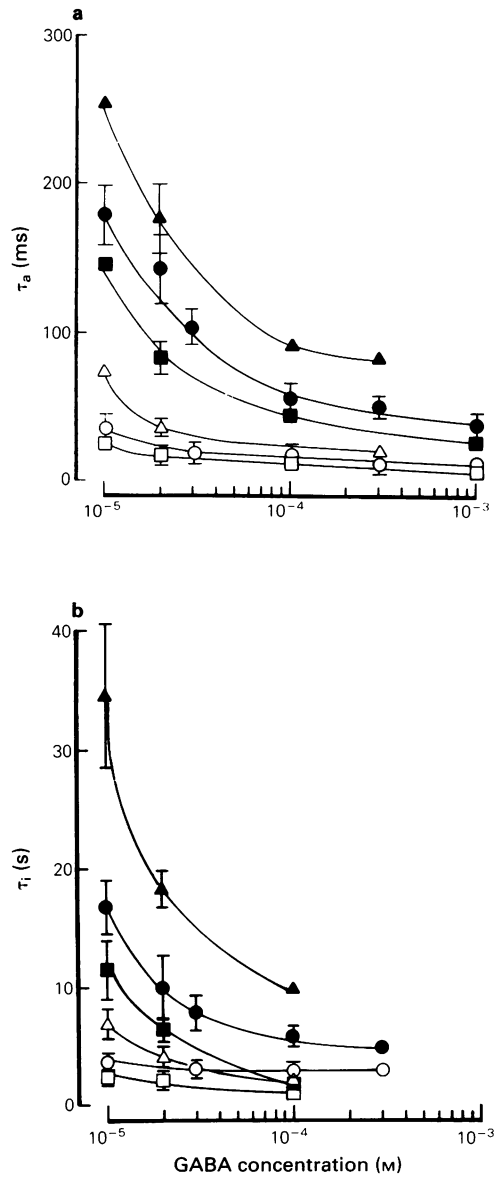

Figure 7 Activation (a) and inactivation (b) time constants as a function of GABA concentration at 10 (△, ▲), 20 (○, ●) and 30 (□, ■) °C. Each point is the mean of 4–7 cells. Vertical lines show s.e.mean where it is larger than the symbol. Both the fast activation (open symbols in (a); τ_{af}) and fast inactivation (open symbols in (b); τ_{if}) were concentration-independent; the slow activation (solid symbols in (a); τ_{as}) and slow inactivation (solid symbols in (b); τ_{is}) were concentration-dependent. Both cooling and heating had a greater effect on τ_{as} and τ_{is} compared to τ_{af} and τ_{if}. The more pronounced effect on activation and inactivation appeared to be at the lower concentration ranges.

heating to 30°C over the GABA concentration range studied (Figure 7a). The slow activation time constant, τ_{as}, decreased with increasing GABA concentration and this effect was enhanced at 30°C. However, in contrast, cooling to 10°C increased τ_{as} (Figure 7a). A similar pattern was observed for the inactivation kinetics (Figure 7b). A small decrease in the fast inactivation time constant, τ_{if}, was observed on heating to 30°C. Similarly, the slow inactivation time constant, τ_{is}, was decreased at 30°C and greatly increased at 10°C (Figure 7b). Thus, over the GABA concentration range examined, both cooling to 10°C and heating to 30°C had a more pronounced effect on the slow activation and inactivation time constants, τ_{as} and τ_{is}, respectively, compared with the fast activation and inactivation time constants, τ_{af} and τ_{if}.

From the whole cell data, it was apparent that the slow time constants rather than the fast time constants of both activation and inactivation, were more sensitive to temperature changes, particularly cooling to 10°C. We therefore examined the effect of cooling to 10°C on the 3×10^{-6} M GABA-gated single channels using 'inside-out' patches ($n = 4$). An example of the effect of cooling to 10°C on the GABA response is shown in Figure 8a. At 10°C, there was a marked increase in the probability of the open state compared to at 20°C (RT). In both the open and closed time histograms of Figure 8b bins were distributed double exponentially. Subtraction of the area under the exponential curve for slow component fit, by computer, from the total histogram gave another exponential distribution with a faster time constant than that of the slow component; this was defined as the fast component. The insert in each histogram of Figure 8b shows the fast component. On cooling to 10°C, the slow and fast components symbolize 657 and 331 events for the open time and 767 and (inset: left, 287 and right, 124) for the closed time, respectively. As shown in the left panels of Figure 8b, both the fast (τ_{of}) and slow (τ_{os}) components of mean open time increased (50 and 26% respectively) with cooling. However, the more pronounced effect of cooling to 10°C was reflected in the mean closed time data. Here, both the fast (τ_{cf}) and slow (τ_{cs}) components of mean closed time were markedly decreased; τ_{cf} was most affected showing a decrease from 57 to 3 ms. However, τ_{cs} also showed a marked decrease from 123 to 54 ms. As shown with this example, the total number of opening events per minute (N_0) increased from 315 at RT to 1023 at 10°C and open probability (P_0) increased from 1.3% at RT to 51% at 10°C. Thus, the probability of the channels being, and remaining open increased on cooling to 10°C. There was, however, no change in the single channel conductance on cooling to 10°C (not shown).

Discussion

These results demonstrate that, in frog sensory neurones, the kinetics of GABA-induced I_{Cl} are temperature-dependent over the temperature range 5–35°C. Temperature-dependent coefficients existed for peak I_{Cl}. On cooling to 10°C, the Q_{10} increased with the highest Q_{10} occurring between 5 and 15°C. Conversely, Q_{10} decreased on heating to 30°C. It is, therefore, apparent that these cells exhibit a greater sensitivity to temperature at the lower temperature range. However, it is also apparent that at 10°C, the affinity of GABA for its receptor is unchanged although its efficacy is reduced (see Figure 3), as shown by an identical Hill number ($n = 2$) and K_d (1.1×10^{-5} M at 20°C and 1.3×10^{-5} M at 10°C). This suggests that the decrease in peak I_{Cl} on cooling to 10°C is not due to changes in binding but subsequent to receptor-channel activation.

A reduction of peak I_{Cl} at low and high temperatures could be explained by a change in the kinetics of channel opening and closing, a decrease in the number of activated channels and/or single channel conductance. One possible factor, besides a reversible reduction in Cl^- conductance, that could have caused a decreased peak I_{Cl} is a reduction of E_{Cl} or Cl^- permeability. However, this was prevented by continuously perfusing the neurone with high external and internal Cl^- and applying a holding potential of between -10 and -15 mV, as described in the Methods section. In contrast, in cat dorsal root ganglion cells, the decrease in peak amplitude of the GABA response on cooling to 10°C was attributed, in part, to a decrease in the driving force generating the GABA-induced current (Gallagher et al., 1983). Here, the GABA potential was shifted to a more negative level (-27 to -19 mV), whereas no appreciable change was observed at the crayfish neuromuscular junction (Onodera & Takeuchi, 1979). In cultured mouse spinal neurones, the equilibrium potential for the GABA-evoked chloride-dependent process was little affected by changes in bath temperature (Mathers & Barker, 1981).

The kinetics of Cl^- channel gating in the cells used in our study appear to be temperature-dependent. Cooling to 10°C preferentially increased the slow activation (τ_{as}) and inactivation (τ_{is}) time constants. Single channel analysis revealed that the greatest effect of cooling to 10°C was to shorten significantly the fast and slow components of the closed time with only small increases in mean open time and an increase in the total number of events. The increase in open probability on cooling to 10°C would be accounted for by the increase in the open event number, which might be due to the slower inactivation time at 10°C. Thus, cooling to 10°C did

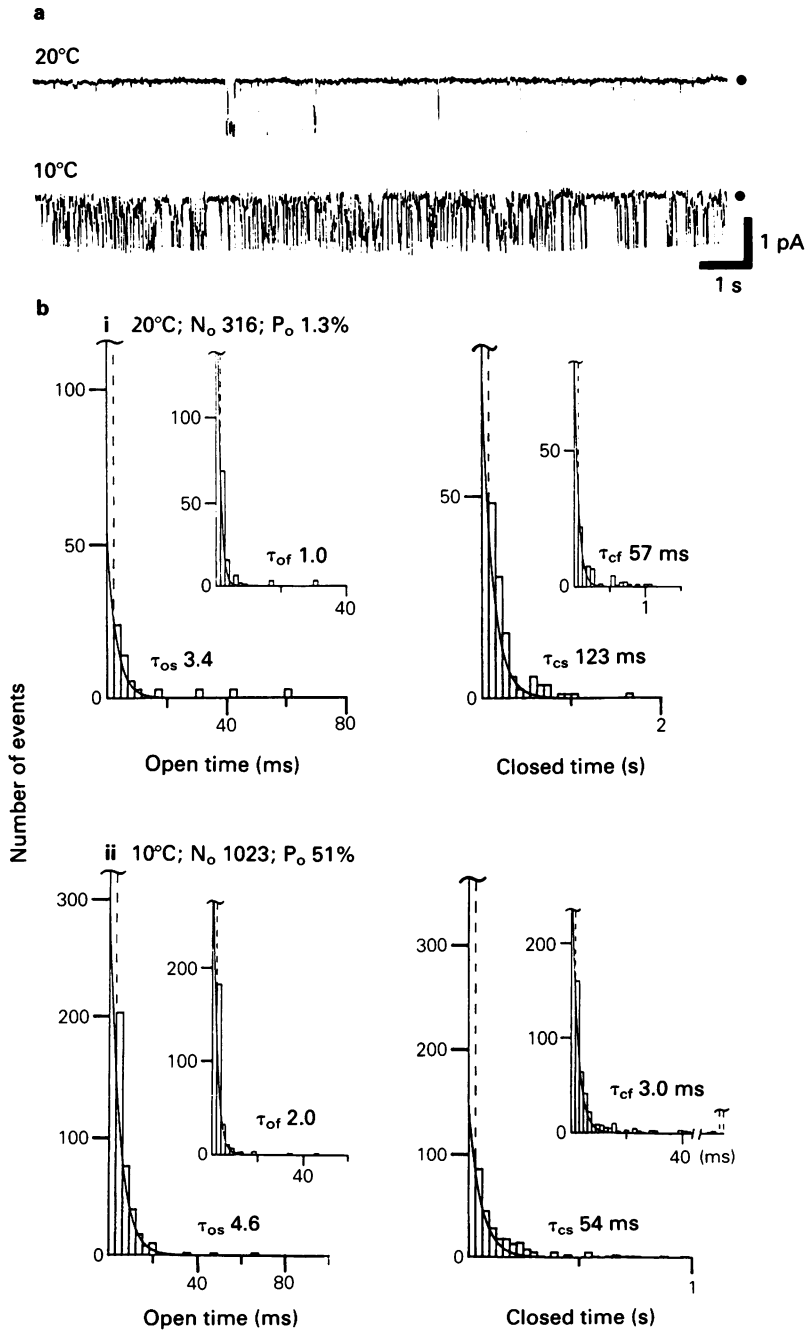

Figure 8 The effect of cooling on the GABA-gated single channel Cl^--current. (a) Single channel record of 3×10^{-6} M GABA at 20°C (upper panel) and 10°C (lower panel). The closed state is indicated by (●) in both upper and lower traces. Note the marked increase in open probability on cooling to 10°C compared to room temperature (20°C). (b) Open and closed time histograms from the same experiment depicted in (a). Upper panel (i): control at 20°C; lower panel (ii): effects of cooling to 10°C. The ordinate scales show the number of events per bin. The inset in each histogram shows the fast component after subtracting the slow component. τ_{of}, fast component of mean open time; τ_{os}, slow component of mean open time; τ_{cf}, fast component of mean closed time; τ_{cs}, slow component of mean closed time; N_0, total number of open events per min and P_0, open probability. See text for further details.

not change the mean open time in the present study. In contrast, the mean open time was found to increase in cultured spinal cord cells, as estimated from the shift in the half-power frequency of the spectrum, f_c, with decreasing temperature (Mathers & Barker, 1981).

In the present study, single channel conductance did not change when decreasing temperature and, hence, could not account for the reduction in peak I_{Cl}. The largest Q_{10} occurred at 10°C which could be an indication of a phase transition of the membrane lipids. A change in the microenvironment of the integral channel proteins may lead to conformational changes in these proteins. A second possible mechanism is a change in membrane thickness which in turn could change the electrical field within the membrane experienced by the activation and inactivation voltage sensors (Onodera & Takeuchi, 1979).

The changes in the gating kinetics of these frog sensory cells on cooling to 10°C could, therefore, be described by a change in molecular structure. However, if this is a valid hypothesis, then the question arises as to whether an assumed phase transition occurs in the membrane lipid components or the Cl channel protein itself.

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